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Optimization and xenobiotics degradation of newly isolated white rot fungi

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ABSTRACT

Two samples were collected from Western Ghats area, of Tamilnadu, South India. The collected fungi were isolated and identified based on the key provided previously. *Phanerochaete chrysosporium* 787 was obtained from Microbial Type Culture Collection, (MTCC) Chandigarh, India and was used as the reference fungus. The isolated fungi were identified and used for the optimization and degradation of 2, 4, dichlorophenoxy acetic acid and the results were analyzed by gas chromatography. In *P. chrysosporium* the maximum growth of the mycelium was found to be 68 mg. In *Schizophyllum commune* it was found to be 66 mg and in *Lenzites eximia it* was reported to be 67 mg. The per cent degradation of 2, 4 dichlorophenoxy acetic acid by *P. chrysosporium* 787 was observed to be 41.7 per cent. Similarly the per cent degradation was 32.6 and 39.0 per cent by *S. commune* and *L. eximia* respectively. The results revealed that *P. chrysosporium* is very efficient for the degradation of 2, 4 dichlorophenoxyacetic acid.

Keywords : White rot fungi; Degradation; Xenobiotic; 2, 4 dichlorophenoxy acetic acid; *Phanerochaete chrysosporium; Schizophyllum commune; Lenzites eximia.*

1. INTRODUCTION

The major environmental problems facing the world today is the contamination of soil, water and air by toxic chemicals. Eighty million pounds of hazardous organopollutats are produced annually in United States and only 10 per cent of these are disposed of safely (Reddy and Mathew, 2001). The production of pesticides started in India in 1952 and now it is the second largest manufacturer of pesticides in Asia and ranks twelfth globally (Marthur 1999). Many xenobiotic compounds have medium to long term stability in soil and their persistence results in significant impact on the soil ecosystem (Gavrilescu, 2005). The term pesticide covers a wide range of compounds including insecticides, fungicides, herbicides, plant growth regulators and others (Aktar et al., 2009). Pesticides are usually applied simultaneously or one after the other and this type of application often leads to a combined contamination of pesticide residues in the soil environment (Chu et al., 2008).

Many polyaromatic hydrocarbons including Polychlorinated dibenzodioxin and dichloro-diphenyltrichloroethane (DDT) are mutagenic and toxic to humans and animals and are important pollutants present in soil and sedimentation. These compounds have long existence period and remain in the environment for a long time (Siripong et al., 2009). One of the most commonly used phenoxyalkanoic herbicide is 2, 4, dichlorophenoxyacetic acid for selective control of weeds and defoliation and they are reported to be mutagenic (Sieler, 1978). Application of pesticides in urban areas is carried out at an extensively high concentration resulting in pesticide waste characterized by prolonged persistence (Khadrani et al., 1999). The physico chemical remedial strategies to clean up sites contaminated by these compounds are not cost effective or adequate enough. Therefore, research is increasingly being focused on biological methods for the degradation and elimination of these pollutants (Jain et al., 2005). Biodegradation refers to the metabolic activity of microorganisms to transform organic contaminants into less harmful compounds (Margesin and Schinner, 2001).

Biodegradation is influenced by many factors such as the microorganism that can degrade the specific chemical structure, environmental conditions that allow the microorganism to grow, express their degradation enzymes, and establish good physical contact between the organic substrate and the organism. Toxic compounds should not be present at high concentrations, since they can inactivate essential enzyme (Martin, 1998). Complete biodegradation results in mineralization of xenobiotic compounds to CO_2 and H_2O (Han et al., 2004).

Microbial metabolism has long been regarded as the most important mechanism of pesticide degradation in soil and it constitutes the basis for all bioremediation strategies. Several conditions that favour microbial growth, activity in soil such as temperature, moisture, nutrient status, pH and aeration will promote metabolic degradation of pesticides. Microorganisms co-metabolize pesticides by oxidation reduction, dehalogenation, ring cleavage and hydrolysis (Gadd, 2001). Sasek (2003) reported that white rot fungi are potentially powerful tools for bioremediation. The application of fungi to clean up contaminated soil first came to attention in the mid 1980's when the white rot fungus *P. chrysosporium* was shown to metabolize a range of organic environmental contaminants (Hernandez et al. 2001).

This ligninolytic system of white rot fungi are directly involved in the degradation of various xenobiotic compounds (Wesenberg, 2003). The degradability of white rot fungi is due to the strong oxidative activity and the low substrate specificity of their ligninolytic enzymes (Ohkuma et al., 2001). The mechanism of biodegradation depends on the compound being degraded. The ligninolytic enzymes in white rot fungi catalyze the degradation of pollutants by

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using a non specific free radical mechanism. When an electron is added or removed from the ground state of a chemical it becomes highly reactive allowing it to give or take it electrons from other chemicals. This provides the basis for the non specificity of the enzymes and the ability to degrade xenobiotics. The main reactions that are catylezed by the ligninolytic enzymes include depolymerization, demethoxylation, decarboxylation, hydroxylation and aromatic ring opening. These reactions result in oxygen activation, creating radicals that facilitate oxidation of organopollutants (Reddy and Mathew, 2001).

Fragoeiro and Magan (2008) reported the successful application of the white rot fungi *T. versicolor* for the bioremediation of mixtures of pesticides in non sterile sandy loam soil under low water potential conditions. Bending et al. (2002) showed 86 per cent degradation of atrazine and terbuthylazine by white rot fungi in liquid culture and found no relationship between degradation rate and ligninolytic activity. *T. versicolor* was used for environmental remediation of pesticides, explosives and industrial solvents (Sasek et al., 2003). The degradation of DDT, lindane and atrazine by *P. chrysosporium* was reported by Reddy and Mathew (2001). The degradation of a variety of environmentally persistent pollutants by *P. chrysosporium* has been reported (Bumpus et al., 1985). Ryu et al. (2000) reported the biodegradation of pentachlorophenol by white rot fungi *Trametes sp.* and *Pleurous sp.* under ligninolytic and non ligninolytic conditions.

2. MATERIALS AND METHODS

2.1. Collection of fungi

The fungi *Schizophyllum commune* and *Lenzites eximia* were collected from Western Ghats area of Tamilnadu, India. *S.commune* and *L.eximia* were isolated from living tree of *Tamarindus indica,* burnt tree respectively. The collection site was situated in the latitude of -11.58,^{o}S and longitude of 76.93^{o}E at 400 ± 50M MSL. It receives rain fall of about 300 mm per year with high humidity and temperature. The reference fungus *Phanerochaete chrysosporium* 787 was obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India and was used for further studies.

2.2. Isolation of the fungi

The portion of the fungi was cut, surface sterilized with 1 per cent mercuric chloride solution and then repeatedly washed with sterile distilled water (Roy Watling, 1971). The fungi were then inoculated on 2 per cent malt agar medium in petriplates. Then the fungal growth which occurred on the plates was sub cultured on malt agar slants to obtain pure culture. The samples were identified based on the morphology of the fruiting bodies and spores based on the key provided previously by (Bakshi 1971; Gilbertson and Ryvarden, 1986).

2.3. Preparation of spore suspension

The fungi were grown in malt agar medium by dissolving 20 g of malt extract and 20 g of agar in distilled water and made up to 1000 ml. The pH was maintained as 6.5 at 37^{o}C. Then the plates were flooded with sterile distilled water and brushed with camel hair brush smoothly without disturbing the mycelial growth and filtered through a sterile filter. The concentration of the filtrate was adjusted to 10^{5}spores/ml and used as inoculums for further studies.

2.4. Growth kinetics and ligninase production

Growth kinetics and ligininase enzyme production were studied in C- Limited medium (M14) of Janshekar and Fiechter (1988).

2.5. Ligninolytic enzymes production

Enzymes, lignin peroxidase (LiP), manganese-dependent peroxidase (MnP) and aryl alcohol oxidase (AAO) were characterized for its ligninolytic enzymes production. The LiP activity was determined using a spectrophotometer (OD at 310 nm) according to the method of Linko (1988). One unit of enzyme activity was defined as the amount of enzyme oxidizing one mole of veratryl alcohol in 1 min. MnP was assayed using a spectrophotometer by the method of Kuwahara et al. (1984). One unit of enzyme activity was expressed as the amount of enzyme capable of increasing one optical density (OD) at 610 nm in a min. The aryl alcohol oxidase activity was determined spectrophotometrically as described by Waldner (1988) with veratryl alcohol as a substrate. In this case, one unit of the enzyme activity was expressed as the amount of enzyme able to increase one OD at 310 nm.

2.6. Determination of mycelial dry weight and enzyme production

Aliquots (50ml) of medium were taken in 250 ml of Erlenmeyer flask and inoculated with 10^{5} spores/ml (inoculums volume - 10%v/v.). The flask was incubated at 30^{o}C. At regular time intervals, the fungal biomass was removed by filtration and the growth was determined as mycelial dry weight (mg) by drying the biomass at 105^{o}C to a constant weight. The culture filtrate was centrifuged at 10,000 rpm for 20 min at 4^{o}C and the clear supernatant was used as enzyme immediately. The boiled culture filtrate was used as enzyme blank.

2.7. Effect of culture parameters on growth and ligninolytic enzyme production

The fungi were grown in C-limited medium (Jansheker and Fiechter, 1988) in orbital shaker (120 rpm) to determine the optimum culture conditions for growth and ligninase production.

2.7.1. Incubation period

The white rot fungi were grown at various incubation periods at room temperature and the mycelial growth and enzyme production were determined at the end of each incubation periods.

2.7.2. pH

The growth and enzyme production were determined at the pH range of 3.0 to 8.0 at room temperature.

2.7.3. Temperature

The effect of temperature was determined in the range of 30 to 80^{o} C.

2.7.4. Carbon sources

Different concentrations of D-glucose, sucrose and cellulose (0 to 4.0%) were used as carbon sources for growth and ligninase enzyme production.

2.7.5. Nitrogen sources

The nitrogenous compounds, di-ammonium tartrate, urea and tryptone at different concentrations (0.22 to 1.1%) were used as nitrogen sources.

2.8. Degradation of Xenobiotics

The degradation of Xenobiotics was carried out in C-Limited medium. The medium was inoculated with fungal spore suspension (10^{5} spores/ml) and incubated at 30^{o}C for 6 days in an orbital shaker. After 6 days the xenobiotics compound 2, 4 Dichlorophenoxy acetic acid (0.25 µM) was added to the medium after dissolved in 0.25N NaOH solution. The cultures were incubated at 37°C on a rotary shaker at 300 rpm for 15 days. The degradation profile of 2, 4 dichlorophenoxy acetic acid was analyzed by gas chromatography. The concentration of 2, 4 dichlorophenoxy acetic acid in the sample was determined by gas chromatography; this was extracted with hexane. 0.5 µL extract was analyzed on a CBP5 fused silica capillary column (25 m × 0.22mm × 0.25 µm; Shimadzu) installed in a Shimadzu GC-14A gas chromatography equipped with FID. Split injection (1:100) and programmable temperature were used with nitrogen as the carrier gas. The oven temperature was 80°C for 1 min, increased to 180°C at a rate of 20°C/min, increased to 220°C at a rate of 4°C/min, increased to 280°C at a rate of 20°C/min, and maintained at 280°C for 5 min. The injection temperature was 250°C and the detector temperature was 250°C. The same procedure was carried out for control without the inoculation of white rot fungus (Ryu et al. 2000).

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Table 1 Effect of incubation period on growth and ligninase production by white rot fungi

Incubation period (days)	Enzyme production (U/ml)								
	LiP			MnP			AAO		
	Pc	Sc	Le	Pc	Sc	Le	Pc	Sc	Le
1	30	35	31	0.01	0.02	0.02	36	43	39
2	39	43	41	0.02	0.04	0.03	75	89	84
3	53	61	57	0.03	0.05	0.03	88	107	104
4	64	71	69	0.04	0.06	0.05	109	118	114
5	87	102	95	0.05	0.09	0.06	120	126	122
6	126	147	143	0.07	0.14	0.12	137	149	145
7	163	177	170	0.09	0.16	0.14	159	190	165
8	151	161	156	0.08	0.14	0.11	141	163	153
9	128	142	137	0.06	0.11	0.09	138	149	144
10	114	127	121	0.05	0.09	0.07	101	138	136
11	82	96	90	0.03	0.07	0.05	87	120	106
12	30	38	35	0.02	0.05	0.03	47	85	66

Pc: *Phanerochaete chrysosporium* 787; Sc: *Schizophyllum commune;* Le: *Lenzites eximia;* LiP: Lignin peroxidase; MnP: Manganese dependent peroxidase; AAO: Aryl alcohol oxidaseValues are mean of three replicates

Table 2 Effect of pH on growth and ligninase production by white rot fungi

pH	Enzyme production (U/ml)								
	LiP			MnP			AAO		
	Pc	Sc	Le	Pc	Sc	Le	Pc	Sc	Le
3.0	54	80	65	0.03	0.04	0.04	42	50	48
3.5	68	95	84	0.04	0.07	0.05	70	99	92
4.0	92	128	101	0.05	0.08	0.06	83	108	105
4.5	126	177	156	0.06	0.09	0.07	114	125	120
5.0	96	146	123	0.05	0.07	0.06	127	144	143
5.5	86	115	108	0.03	0.06	0.05	150	174	170
6.0	51	82	75	0.02	0.05	0.03	165	194	185
6.5	35	40	37	0.01	0.03	0.02	129	153	150
7.0	ND	ND	ND	ND	ND	ND	8	34	20
7.5	ND	ND	ND	ND	ND	ND	ND	ND	ND
8.0	ND	ND	ND	ND	ND	ND	ND	ND	ND
8.5	ND	ND	ND	ND	ND	ND	ND	ND	ND

Pc: *Phanerochaete chrysosporium* 787; Sc: *Schizophyllum commune;* Le: *Lenzites eximia;* LiP: Lignin peroxidase; MnP: Maganese dependent peroxidase; AAO: Aryl alcohol oxidase Values are mean of three replicates

Table 3 Effect of temperature on growth and ligninase production by white rot fungi

Temperature° C	Enzyme production (U/ml)								
	LiP			MnP			AAO		
	Pc	Sc	Le	Pc	Sc	Le	Pc	Sc	Le
30	41	59	62	0.02	0.03	0.04	25	28	34
35	83	108	111	0.03	0.04	0.07	85	91	102
40	144	152	170	0.06	0.08	0.09	152	163	181
45	123	143	154	0.05	0.07	0.08	126	147	166
50	85	89	91	0.04	0.06	0.07	71	93	107
55	42	47	55	0.03	0.04	0.05	64	77	85
60	38	42	45	0.02	0.03	0.04	47	67	82
65	32	40	42	0.02	0.02	0.03	36	38	40
70	24	31	36	0.01	0.02	0.02	7.0	18	23

Pc: *Phanerochaete chrysosporium* 787; Sc: *Schizophyllum commune;* Le: *Lenzites eximia;* LiP: Lignin peroxidase; MnP: Maganese dependent peroxidase; AAO: Aryl alcohol oxidase Values are mean of three replicates

Table 4a Effect of carbon sources on growth and ligninase production by *Phanerochaete chrysosporium* 787

Substrate concentration (g/l)	Growth (Mycelial dry weight in mg)	Enzyme production (U/ml)		
		LiP	MnP	AAO
Control	10.0	7	0.01	12
Glucose 2.0	25.8	80	0.11	54
2.5	27.3	92	0.11	87
3.0	29.0	91	0.14	127
3.5	30.0	146	0.12	143
4.0	25.3	94	0.01	109
Cellulose 2.0	23.2	73	0.02	54
2.5	25.7	111	0.03	67
3.0	27.3	126	0.05	96
3.5	30.0	135	0.03	140
4.0	24.3	87	0.02	108
Lignin 2.0	21.7	76	0.07	38
2.5	23.6	110	0.08	79
3.0	29.0	124	0.11	117
3.5	32.0	142	0.10	148
4.0	27.0	110	0.09	103

LiP : Lignin peroxidase; MnP: Manganese dependant peroxidase; AAO: Aryl alcohol oxidase, Values are mean of three replicates

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Table 4b Effect of carbon sources on growth and ligninase production by *Schizophyllum commune*

Substrate concentration (g/l)	Growth (Mycelial dry weight in mg)	Enzyme production (U/ml)		
		LiP	MnP	AAO
Control	12.0	10	0.02	14
Glucose 2.0	32.5	101	0.15	90
2.5	34.0	140	0.16	142
3.0	35.7	192	0.17	171
3.5	42.0	205	0.16	187
4.0	32.4	149	0.13	141
Cellulose 2.0	30.4	87	0.03	75
2.5	32.2	128	0.06	95
3.0	34.4	156	0.09	135
3.5	38.1	160	0.07	119
4.0	36.6	126	0.03	90
Lignin 2.0	30.4	95	0.09	95
2.5	32.4	128	0.10	96
3.0	37.4	206	0.14	160
3.5	40.6	184	0.12	195
4.0	36.3	140	0.10	152

LiP : Lignin peroxidase; MnP: Manganese dependant peroxidase; AAO: Aryl alcohol oxidase, Values are mean of three replicates

Table 4c Effect of carbon sources on growth and ligninase production by *Lenzites eximia*

Substrate concentration (g/l)	Growth (Mycelial dry weight in mg)	Enzyme production (U/ml)		
		LiP	MnP	AAO
Control	11.0	9	0.01	16
Glucose 2.0	30.6	94	0.15	83
2.5	32.5	124	0.17	104
3.0	33.0	154	0.18	145
3.5	35.6	176	0.17	178
4.0	30.7	123	0.16	124
Cellulose 2.0	24.3	79	0.03	78
2.5	28.4	118	0.05	113
3.0	30.6	153	0.07	133
3.5	33.6	155	0.06	154
4.0	31.1	93	0.02	116
Lignin 2.0	28.6	65	0.08	75
2.5	30.5	118	0.09	84
3.0	36.4	146	0.12	142
3.5	39.4	160	0.10	179
4.0	35.3	126	0.09	105

LiP : Lignin peroxidase; MnP: Manganese dependant peroxidase; AAO: Aryl alcohol oxidase, Values are mean of three replicates

Table 5a Effect of nitrogen sources on growth and ligninase production by *Phanerochaete chrysosporium* 787

Substrate concentration (g/l)	Growth (Mycelial dry weight in mg)	Enzyme production (U/ml)		
		LiP	MnP	AAO
Control	10.0	17	0.01	20
Diammonium tartrate 0.22	27.0	69	0.03	77
0.44	29.0	141	0.04	136
0.66	32.0	153	0.08	144
0.88	31.0	132	0.07	52
1.10	28.6	85	0.04	76
Urea 0.22	24.0	71	0.04	89
0.44	29.3	129	0.05	104
0.66	30.4	97	0.06	131
0.88	27.6	65	0.03	109
1.10	24.3	39	0.03	66
Tryptone 0.22	26.3	41	0.03	74
0.44	30.3	144	0.04	137
0.66	31.5	95	0.05	109
0.88	27.3	77	0.04	84
1.10	25.0	47	0.02	47

LiP : Lignin peroxidase; MnP: Manganese dependant peroxidase; AAO : Aryl alcohol oxidase, Values are mean of three replicates

3. RESULTS AND DISCUSSION

3.1. Effect of culture condition on growth and ligninase production

3.1.1. Incubation period

The effect of incubation period on culture condition and ligninase production by *P. chrysosporium* 787, *S. commune* and *L. eximia* were presented in Table 1. The results showed that maximum growth was observed by *P. chrysosporium* on eleventh and twelfth day (35.6 mg mycelial dry weight). Initial production of LiP on first day was 30 U/ml; it has increased up to 163 U/ml on seventh day of incubation period. Initially MnP production by *P. chrysosporium* was 0.01 U/ml and maximum production was observed on seventh day (0.09 U/ml). On the first day, the aryl alcohol oxidase enzyme production was 36 U/ml, the maximum production (159 U/ml) was achieved on seventh day, after that the enzyme production was reduced. The entire three enzymes had maximum activity on seventh day and then the activity was decreased. In *S. commune* maximum growth (46.0 mg mycelial dry weight) was observed on twelfth day of incubation. LiP (177 U/ml), MnP (0.16 U/ml) and AAO (190 U/ml) were maximum on seventh day. In *L. eximia* maximum growth (44.0 mg mycelial dry weight) was observed on twelfth day; LiP, MnP and AAO production were maximum on seventh day and it was found

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Table 5b Effect of nitrogen sources on growth and ligninase production by *Schizophyllum commune*

Substrate concentration (g/l)	Growth (Mycelial dry weight in mg)	Enzyme production (U/ml)		
		LiP	MnP	AAO
Control	16.0	20	0.02	22
Diammonium tartrate 0.22	33.4	95	0.05	137
0.44	36.6	196	0.08	146
0.66	46.2	176	0.10	176
0.88	35.4	145	0.06	168
1.10	34.0	107	0.03	159
Urea 0.22	29.3	86	0.04	98
0.44	33.6	150	0.06	168
0.66	34.8	169	0.08	186
0.88	33.3	144	0.07	148
1.10	30.0	97	0.03	106
Tryptone 0.22	29.0	59	0.03	87
0.44	36.3	78	0.04	172
0.66	37.0	158	0.07	180
0.88	34.0	141	0.06	126
1.10	33.0	92	0.03	79

LiP : Lignin peroxidase; MnP: Manganese dependant peroxidase; AAO: Aryl alcohol oxidase, Values are mean of three replicates

Table 5c Effect of nitrogen sources on growth and ligninase production by *Lenzites eximia*

Substrate concentration (g/l)	Growth (Mycelial dry weight in mg)	Enzyme production (U/ml)		
		LiP	MnP	AAO
Control	14.0	19	0.01	21
Diammonium tartrate 0.22	30.5	80	0.04	105
0.44	32.4	156	0.05	133
0.66	34.0	171	0.08	168
0.88	31.0	138	0.06	146
1.10	31.0	106	0.04	131
Urea 0.22	26.0	90	0.04	94
0.44	31.0	163	0.05	156
0.66	32.0	183	0.07	150
0.88	30.0	155	0.04	130
1.10	26.0	110	0.03	87
Tryptone 0.22	29.0	41	0.02	84
0.44	33.4	154	0.03	126
0.66	35.0	140	0.04	157
0.88	32.0	96	0.03	107
1.10	30.3	77	0.02	63

LiP : Lignin peroxidase; MnP: Manganese dependant peroxidase; AAO: Aryl alcohol oxidase, Values are mean of three replicates

Table 6 Biodegradation of xenobiotic by white rot fungi

Fungi	Incubation period (days)	Mycelial dry weight (mg)	Per cent degradation
Control	15	-	-
P. chrysosporium 787	15-68	41.7	
S. commune	15	66	32.6
L. eximia	15-67	39.0	

to be 170 U/ml, 0.14 U/ml and 165 U/ml. After the optimum period the productions were gradually decreased. Patrick et al. (2011) have reported that the optimum temperature for maximum laccase and MnP production by *P. sajor-caju* was found to be 30°C on day 9 with an activity of 0.2844 U/ml and 20°C on day 7 with an activity of 0.0052 U/ml, respectively. Very little lignolytic activities were observed at temperatures above 30°C probably due to the fact that increasing the temperature could have inhibited the fungal growth and hence, low/decreased enzyme activities. The same trend has also been demonstrated by Zadrazil et al. (1999) when *Pleurotus* species and *Dichomitus squalens* were cultivated at temperatures higher than 30°C.

3.1.2 pH

The effect of pH on culture conditions and ligninase production were studied in the pH range from 3.0 to 8.5. The results revealed (Table 2) that, in *P. chrysosporium* 787 maximum (35.0 mg dry weight) growth was observed at pH 4.5, LiP production and MnP Production were also favoured (126 U/ml; 0.06 U/ml) at pH 4.5. At pH 6.0 maximum level of AAO was produced (165 U/ml). In *S. commune*, maximum growth (41.3 mg dry weight) was observed at pH 4.5; LiP, MnP and were maximum at pH 4.5 and the production was found to be 177.0, 0.09. For AAO maximum enzyme production 194 U/ml was observed on pH 6.0. In *L. eximia*, maximum growth (37.8 mg dry weight) was observed at pH 4.5. LiP, MnP productions were maximum at pH 4.5, AAO production was favoured at a pH of 6.5. LiP MnP was produced to a maximum of 156 U/ml and 0.07 U/ml respectively. In AAO maximum production was observed up to 185 U/ml at pH 6.0. Maximum laccase and MnP produced were 0.300 and 0.0074 U/ml, respectively at pH 6.0 (day 7) and 5.5 (day 9), respectively. Activities in the most acidic medium (pH 3.5) were low compared to slightly acidic medium (Iqbal et al. 2011).

3.1.3. Temperature

In the present study the effect of temperature on culture condition and ligninase production was studied in the temperature ranges from 30 to 70°C. The results (Table 3) showed that, 40°C favoured mycelial growth (18.0 mg dry weight) of *P. chrysosporium* 787. LiP, MnP and AAO productions was favoured at 40°C and were found to be 144 U/ml, 0.06 U/ml and 152 U/ml respectively. In *S. commune*, mycelial growth (20.3 mg dry weight) was favoured at 40°C. At 40°C, LiP, MnP and AAO were produced to a maximum of 152 U/ml, 0.08 U/ml and 163 U/ml respectively. In *L. eximia* mycelial growth (34.3 mg dry weight) was favoured at 45°C. The LiP, MnP and AAO enzyme productions was found to be maximum at 170 U/ml, 0.09 U/ml and 181 U/ml respectively. Nakamura et al. (1999)

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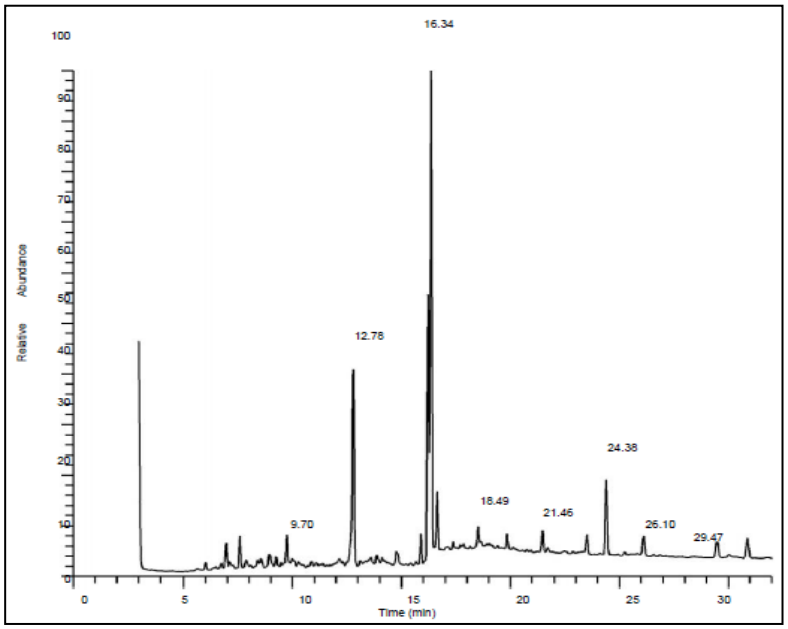

Figure 1

Biodegradation of xenobiotic by control, Compound name: 2, 4-Dichlorophenoxy acetic acid, Molecular formula: C8H6Cl2O3, Peak Area: 547298.83

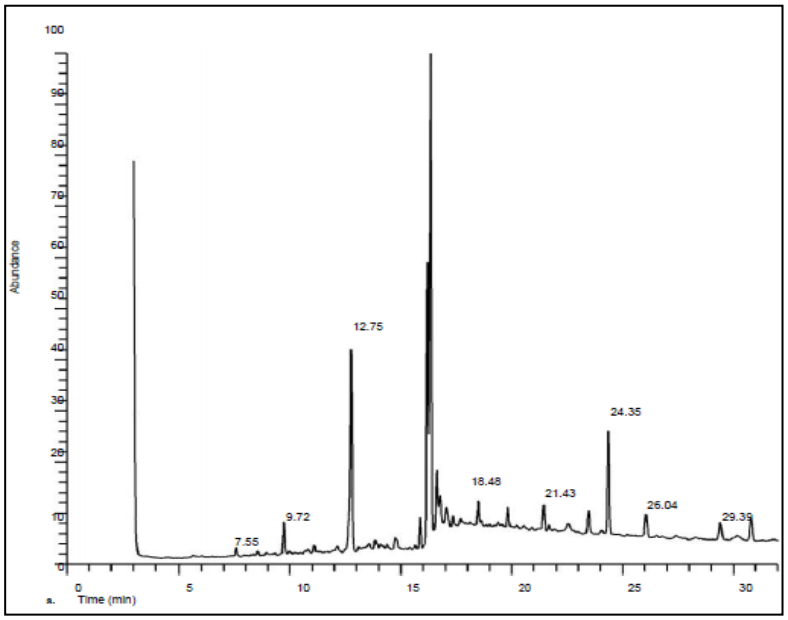

Figure 2

Biodegradation of xenobiotic by *P. chrysosporium* 787, Compound name : 2,4-Dichlorophenoxy acetic acid, Molecular formula : C8H6Cl2O3, Peak area : 8766.54

reported, maximum lignolytic activity from cultures of *Badusta* were attained at 30°C, but above 37°C there was no activity observed. Also, Iqbal et al. (2011) found substantial decrease in ligninolytic enzymes of *Trametes versicolor* IBL-04 when cultivated at temperatures higher than 30°C.

3.1.4. Carbon sources

The mycelial growth for *P. chrysosporium* 787, *S. commune* and *L. eximia* were favoured at 3.5 g/l for amendments of glucose, cellulose and lignin. The carbon source glucose at 3.5 g/l, 3.0 g/l and lignin 3.5 g/l concentration favoured LiP, MnP and AAO production respectively in *P. chrysosporium* 787. In *S. commune* lignin at 3.0g/l favoured LiP production, glucose 3.0 g/l and lignin 3.5 g/l favoured MnP and AAO production respectively. For *L. eximia* glucose at 3.5 g/l favoured LiP production, glucose at 3.0 g/l l favoured MnP and lignin at 3.5g/l favoured AAO production respectively. The optimum carbon sources for ligninase production were determined (Table 4). Patrick et al. (2011) reported, the enzyme production increased with increase in the glucose or cellulose concentration up to 10 g/L at which their maximum values were reached and then declined starting at a concentration of 15 g/L. For manganese peroxidase production, glycerol (2 to 10 g/L) and cellulose (2 to 20 g/L) were used as carbon sources. 2 g/L of cellulose and 4 g/L glycerol were found to be the optimum amounts required for maximum MnP production (0.91 and 0.33 U/ml, respectively).

3.1.5. Nitrogen sources

Among the nitrogen sources, diammonium tartrate at 0.66 g/l concentration favoured mycelial growth of *P. chrysosporium* 787. But LiP, MnP and AAO production were favoured at 0.66 g/l of diammonium tartrate. The optimum mycelial growth of *S. commune* was obtained at 0.66 g/l of diammonium tartrate with maximum production of LiP and MnP at 0.44 g/l and 0.66 g/l concentration of diammonium tartrate whereas AAO was favoured at 0.66 g/l, concentration of urea. For *L. eximia* maximum mycelial growth was favoured at 0.66 g/l of tryptone. LiP was found to be maximum at a concentration of 0.66g/l of Urea, MnP and AAO was favoured at a maximum concentration of 0.66 g/l of diammonium tartrate respectively.The optimum nitrogen source for ligninase production showed in Table 4.

3.2. Xenobiotic degradation by white rot fungi

In the present study degradation of 2, 4 dichlorophenoxy acetic acid by white rot fungi was analyzed by gas chromatography and the results are tabulated in Table 5. In *P. chrysosporium* the maximum growth of the mycelium was found to be 68 mg. In *S. commune,* it was found to be 66 mg and in *L. eximia* it was reported to be 67 mg. The per cent degradation of 2, 4 dichlorophenoxy acetic acid by *P. chrysosporium* 787 was observed to be 41.7 per cent (Fig. 2). Similarly the per cent degradation was 32.6 and 39.0 per cent by *S. commune* and *L. eximia* respectively (Fig 3 and Fig 4). Biodegradation of xenobiotic compounds depends on their concentration, pH of the medium, temperature, availability of water and other nutrients and presence of organic compounds (Fig.1, Table 6). Bioremediation is defined as the application of biological processes to the treatment of pollution. Recently Mycoremediation plays an important role in the degradation of xenobiotics (Aislabe et al., 2007). The ligninolytic fungi degrade a wide variety of organopollutants such as chlorophenols, chlorolanilines, pesticides such as 2, 4, Dicholorophenoxy acetic acid, DDT, methoxychloro and polyaromatic hydrocarbons. These pollutants are co-oxidized by the fungi to give CO_2 and largely uncharacterized polar metabolites. The xenobiotic oxidations by white rot fungi are rapid, efficient and non specific and this property allows them to degrade a large number of xenobiotics. Biological decomposition of pesticides is the most important and effective way to remove these compounds from the environment. Microorganisms have the ability to interact, both chemically and physically, with substances leading to structural changes or complete degradation of the target molecule. Verdin et al. (2004) reported 32 per cent degradation of fluroanthene by the white rot fungi *P. chrysosporium.* Several fungi, such as *Agrocybe semiorbicularis*, *Auricularia auricula*, *Coriolus versicolor*, *Dichomitus squalens*, *Flammulina velupites*, *Hypholoma fasciculare*, *Pleurotus ostreatus*, *Stereum hirsutum,* and *A. discolor,* have shown their ability to degrade various pesticide groups like phenylamide, triazine, phenylurea, dicarboximide, chlorinated and organophosphorus compounds (Bending et al., 2002). *P. chysosporium* degraded isoproturon belonging to phenylurea groups (Wiren et al., 2001), *B. adusta* and *A. discolor* Sp4 degraded the pesticide pentachlorophenol (Rubilar et al., 2007) and *T. versicolor* and *Agaricus augustus* degraded 33 per cent of 2, 4, 6-tribromophenol (Donoso et al., 2008). In the present study degradation of 2, 4 dichlorophenoxy acetic acid by white rot fungi was analyzed by gas chromatography and the results showed

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that maximum degradation was found in *P. chrysosporium* 41.7 per cent when compared to *S.commune and L.eximia.*

4. CONCLUSION

In the present study the effect of culture conditions such as Incubation period, pH, Temperature, Carbon source and Nitrogen source) of fungal growth and ligninolytic enzyme production were studied in C-limited medium (M14) of Janshekar and Fiechter (1988). The enzymes studied were Lignin peroxidase (LiP), Manganese Dependant Peroxidase (MnP) and Aryl alcohol oxidase. Biodegradation of xenobiotic compound 2, 4 dichlorophenoxy acetic acid by white rot fungi *P.chrysosporium* have successfully degraded up to 41.7 percent.

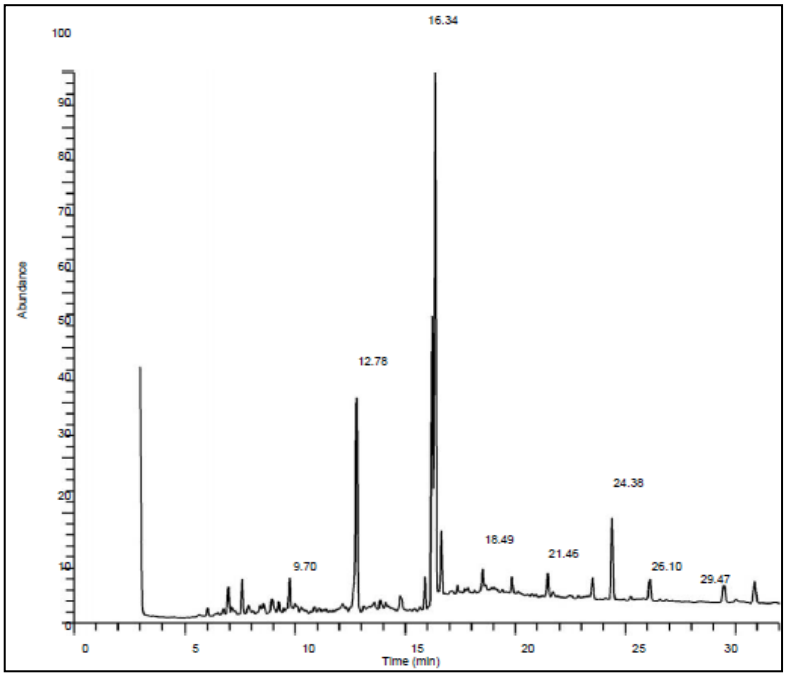

Figure 3

Biodegradation of xenobiotic by *S. commune,* Compound name: 2,4-Dichlorophenoxy acetic acid, Molecular formula : C8H6Cl2O3, Peak area : 333311.89

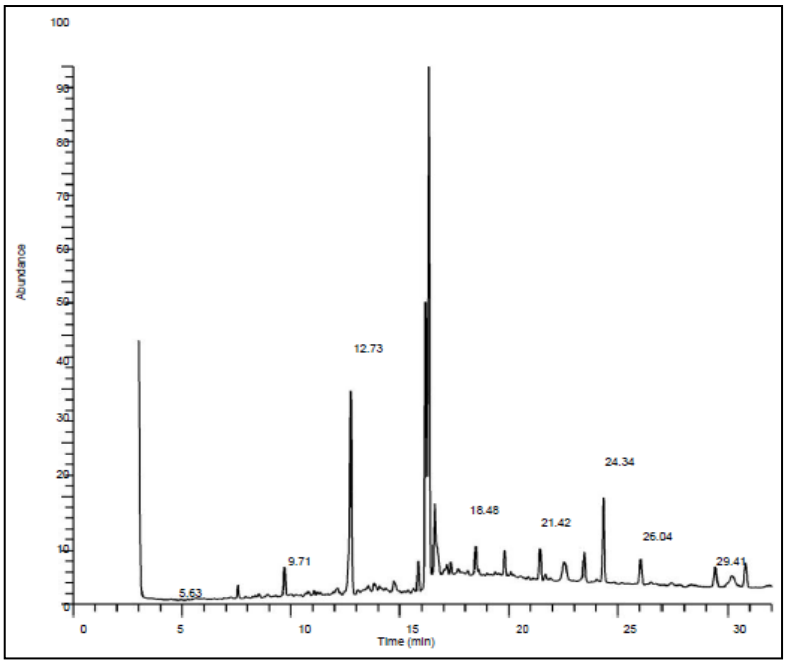

Figure 4

Biodegradation of xenobiotic by *L.eximia,* Compound name: 2, 4-Dichlorophenoxy acetic acid, Molecular formula: C8H6Cl2O3, Peak area: 356500.07

REFERENCES

1. Aislabie JM, Richard K, Boul L. Microbial degradation of DDT and its residues- A Review. *J Agricul Res*., 2007, 40, 269-282
2. Aktar W, Sengupta D, Chowdhury A. Impact of pesticide use in agriculture, their benefits and hazards. *Interdisciplinary Toxicol*. 2009, 2, 1-12
3. Bakshi BK. Indian polyporaceae - on trees and timbers, Indian Council for Agricultural Research ICAR Publication, New Delhi. 1971
4. Bending G, Friloux DM, Walker A. Degradation of contrasting pesticides by white rot fungi and its relationship with ligninolytic potential. *FEMS Microbiol Lett*., 2002, 212, 59-63
5. Bumpus JA, Tien M, Wright D, Aust SD. Oxidation of persistent environmental pollutants by a white rot fungus. *Science*, 1985, 228, 1434-1436
6. Chu X, Fang H, Pan X, Shan WX, Feng M, Yu Y. Degradation of chlorpyrifos alone and in combination with chlorothalonil and their effects on soil microbial populations. *J Environ Sci*., 2008, 20, 464-49
7. Donoso C, Becerra J, Martinez M, Garrido N, Silva M. Degradative ability of 2, 4,6-tribromophenol by saprophytic fungi *Trametes versicolor* and *Agaricus augustus* isolated from Chilean forestry. *World J Microb Biotechnol*., 2008, 24, 961-968
8. Fragoeiro S, Magan N. Impact of *Trametes versicolor* and *Phanerochaete chrysosporium* on differential breakdown of pesticide mixtures in soil microcosms at two water potentials and associated respiration and enzyme activity. *Int Biodeter Biodegrad*., 2008, 62, 376-383
9. Gadd K. Use of spent mushroom compost to bioremediate PAH contaminated samples. *Chemosphere*, 2001, 52, 1539-46
10. Gilbertson RL, Ryvarden L. North American polypores, vol. J Fungiflora, Gronlands Grafiske A/S Oslo, Norway, 1986, 433
11. Gravilescu M. Fate of pesticides in the environment and its bioremediation. *Eng Life Sci*., 2005, 5, 497-526
12. Han MJ, Choi HT, Song HG. Degradation of phenanthrene by *Trametes versicolor* and its laccase. *Journal of Microbiology*, 2004, 42, 94–98
13. Hassan DG, Nadeem A, Ganai A, Muzafer A, Farooq BA, Sofi TA. Biodiversity of macrofungi from conifer dominated forests of Kashmir, *India. J Mycol Plant Pathol*, 2010, 40, 169-175
14. Hernandez TMR, Munguia LA, Ramirez RQ. Residual compost of *Agaricus bisporus* as a source of crude laccase for enzymic oxidation of phenolic compounds. *Process Biochem*. 2001, 76, 113-117
15. Iqbal HMN, Asgher M, Bhatti HN. Optimization of physical and nutritional factors for synthesis of lignin degrading enzymes by novel strain of *Trametes versicolor*. *Bioresource*, 2011, 6, 1273-1287

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Species, 2013, 3(7), 4-11,

16. Jain R, Kapur1 KM, Labana S, Lal B, Sarma PM, Bhattacharya D, Thakur IS. Microbial diversity, Application of microorganisms for the biodegradation of xenobiotics. *Current Sci*. 2005, 89, 101-112
17. Jansekhar H, Fietchter A. Cultivation of *Phanerochaete chrysosporium* and production of lignin peroxidase in submerged stirred tank reactors. *J Biotechnol*. 1988, 8**,** 97-112
18. Khadrani A, Murandi F, Steinman R, Vrousami T. Degradation of three phenylurea herbicides Chlorotulun, isoproturon and diuron by micromycetes isolated from soil. *Chemosphere*, 1999, 38, 3041-50
19. Kuwahara M, Glenn JK, Morgan MA, Gold MH. Separation and characterization of two extracellular H_2O_2 dependent oxidases from ligninolytic cultures of *Phanerochaete chrysosporium*. *FEBS Lett*. 1984, 169**,** 247-250
20. Linko S. Continuous production of lignin peroxidase by immobilized *Phanerochaete chrysosporium* in a pilot scale bioreactor. *J Biotechnol*. 1988, 8**,** 163-170
21. Margesin R, Schinner F. Biodegradation and bioremediation of hydrocarbons in extreme environments. *Appl Microbiol Biotechnol*. 2001, 56, 650–663
22. Marthur SC. Future of Indian pesticide industry in next millennium. *Pesticide Information*, 1999, 24, 9-23
23. Martin J. The aryl hydrocarbon receptor complex. *Ann Rev Pharmacol Toxicol*., 1998, 35, 307-340
24. Nakamura Y, Mtui GYS, Sawada T, Kuwahara M. Lignin degrading enzyme production from *Bjerkandera adusta* immobilized on polyurethane foam. *J Biosci Bioeng*. 1999, 88, 41-47
25. Ohkuma M, Yoshimasa M, Johjima T, Kudo T. Lignin degradation and roles of white rot fungi, Study on an efficient symbiotic system in fungus growing termites and its application to bioremediation. *Riken Review*, 2001, 42**,** 39-42
26. Patrick F, Mtui G, Mshandete AM, Kivaisi A. Optimization of laccase and manganese peroxidase production in submerged culture of *Pleurotus sajorcaju. African Journal of Biotechnology*, 2011, 10, 10166-10177
27. Reddy CA, Mathew Z. Bioremediation potential of white rot fungi in bioremediation. GM Gadd Cambridge, UK Cambridge University Press, 2001
28. Rubilar O, Feijoo G, Diez MC, Lu Chau TA, Moreira MT, Lema JM. Biodegradation of pentachlorophenol in soil slurry cultures by *Bjerkandera adusta* and *Anthracophyllum discolor*. *Indust Eng Chem. Res*., 2007, 46, 744-751
29. Ryu WR, Shim SH, Jeon MYJ, Oh KK, Cho MH. Biodegradation of pentachlorophenol by white rot fungi under ligninolytic and nonligninolytic conditions. *Biotechnol Bioprocess Eng*, 2000, 5, 211-214
30. Sasek S. The utilization of bioremediation to reduce soil contamination, Problems and solutions, Kluwer Academics Publishers, 2003, 247-256
31. Sieler K . Hormones and resistance. *J Pharm*. 1978, Sc 60, 1-6
32. Siripong PB, Oraphin T, Duanporn P. Screening of fungi from natural sources of Thailand for degradation of Polychlorinated hydrocarbons. American Eurasian. *J Agric Environ Sci*. 2009, 5, 466-472
33. Verdin AA, Sahraoui LH, Durand R. Degradation of benzopyrene by mitosporic fungi and extracellular oxidative enzymes. *Int Biodeter Biodegrad*., 2004, 53**,** 65-70
34. Waldner R, Leisola MSA, Fiechter. A comparision of ligninolytic activities of selected white rot fungi. *Appl Microbiol Biotechnol*., 1988, 29**,** 400-407
35. Watling R. Basiodiomycetes, Homobasidiomycetidae. In, Booth C Ed Methods in Microbiology, V.4, Academic press, London and New York, pp, 1971, 219–236
36. Wesenberg D, Kyriakides I, Spiros N, Agathos. White-rot fungi and their enzymes for the treatment of industrial dye effluents. *Biotechnology advances*, 2003, 22,161-187
37. Wiren L, Castillo S, Torstensson MP, Scheunert LI. Degradation of isoproturon in biobeds. *Biol Fert Soils*, 2001, 33, 535-540
38. Zadrazil F, Gonser A, Lang E. Influence of incubation temperature on the secretion of extracellular ligninolytic enzymes of *Pleurotus sp*. and *Dichomitus squalens* into soil. Proceedings of the conference on Enzymes in the environment. Activity, Ecol Applications Granada, Spain, p. 1999, 526

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